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REMARKS/ARGUMENTS

Reconsideration of this application is requested. Claims 1-17 are in the case.

I. NON-COMPLIANT AMENDMENT

In response to the Notice of Non-Compliant Amendment mailed with the outstanding Action, the amendment requested at page 32, beginning at line 25 has been corrected to show the SEQ ID identifiers previously presented with the Amendment dated August 16, 2006. No new matter is entered.

II. THE RESTRICTION REQUIREMENT

It is noted, with appreciation, that Groups II, III and IV have been rejoined.

III. SPECIFICATION

Objection has been made to the presence of improperly identified trademarks. In response, the specification has been amended to incorporate the appropriate demarcations for FACScalibur®, CellQuest®, Trizol®, SuperScript® II, Amplitaq® and MetaPhor®.

Objection has been raised to the presence of hyperlinks in the specification. In response, the specification has been amended to omit the SAGE website reference (www.ncbi.nlm.nih.gov/SAGE) and replace with the following reference: Lash *et al.*, 2000, *Genome Res.* 10:1051-1060. No new matter is entered.

The Action notes that incorrect page numbers are cited for the Church *et al.* reference. The reference has been amended to read: Church, W.B. et al. (2001), *J. Biol. Chem.* 276: 33156-33164.

Withdrawal of the formal objections to the specification is now respectfully requested.

IV. PRIORITY

The Action asserts that claims 1-3 and 5-10 are not entitled to the priority date of the priority application because they do not meet the written description or enablement requirements of US law. In response, and without conceding to this assertion, the claims as amended herein are entitled to the earliest priority date for reasons detailed below.

The Action additionally asserts that the present application discloses features, such as androgen-independent prostate cancer (AIPC) cells, that are not disclosed in the priority application. This objection relates mainly to claim 3 which specifies that the cells are AIPCs. However, it is noted that the underlying PCT International Application AU03/00719 filed June 10, 2003 does disclose AIPCs. Claim 3 is therefore entitled at least to the June 10, 2003 filing date of the underlying '719 PCT International Application.

V. THE 35 U.S.C. §112, SECOND PARAGRAPH, REJECTIONS

Claims 1-3 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. This rejection is respectfully traversed.

The Action asserts that the term "PLA₂ inhibitor" is too broad. The Action further asserts that, given the broad range of PLA₂ enzymes known to exist, the term should be defined in claims 1-3 by reference to a specific PLA₂ polypeptide sequence.

In response, claim 1 has been amended method of inhibiting or reducing the proliferation of prostate cancer cells that express sPLA₂-IIA by administering to the cells a selective inhibitor of sPLA₂. Claim 2 has been amended to claim a method for the treatment of prostate cancer by administering to a subject diagnosed with prostate cancer and requiring treatment for the cancer a selective inhibitor of sPLA₂-IIA inhibitor, wherein the prostate cancer is due to the presence of prostate cancer cells that express sPLA₂-IIA. Specific sPLA₂-IIA sequences are also provided in the sequence listing of the specification.

Claims 1-3 and 5-10 stand rejected under 35 U.S.C. §112, second paragraph, for the reasons beginning on page 7 of the Action. In particular, objection is made to the term "sPLA₂-IIA" in claims 5 - 10 as not clearly defining the polypeptide and it is alleged that the claims do not define the specific isoforms of sPLA₂-IIA that are inhibited by the peptide inhibitors. The Action suggests that these claims include reference to a specific sPLA₂-IIA sequence and to the *specific* activity which must be inhibited to help overcome the objection. The Action also asserts in Item 14(c) that *Markova et al.* describe variants of sPLA₂-IIA which have disparate activities and that the current claims do not therefore clearly identify the relevant sPLA₂-IIA polypeptides that should be targeted to achieve reduction in proliferation of prostate cancer cells.

It is noted at the outset that many of the comments and citations in the Action are drawn from *animal models of colon cancer* and their cells lines. These do not relate to

GRAHAM et al
Appl. No. 10/517,256
January 16, 2009

prostate cancer because, as taught in *Fijneman et al.*, Section 3.5, paragraph 1 (cited in the Action at page 17, paragraph 3) and the reference cited therein (*Belinksi et al.*, copy of Abstract attached), the effects described in *Markova et al.* are tissue and cell line dependent. Thus, the findings regarding sPLA₂-IIA variants cannot be applied to the present invention. Furthermore, there is little evidence that variants of sPLA₂-IIA have any impact on human colon cancer (see particularly *Tomlinson et al.*, copy of Abstract attached), thereby making the mouse data in *Markova et al.* even less relevant to human prostate cancer. Further, Applicants are not aware of any publicly available data indicating that sPLA₂-IIA variants have been identified in association with prostate cancer.

In light of the above, it would be clear to one of ordinary skill that the activity which must be inhibited is the sPLA₂-IIA-mediated proliferation of prostate cancer cells. This is particularly clear in light of the disclosure at page 26, lines 1-9 and elsewhere throughout the present specification.

The Action objects to the phrase "in need thereof" in claim 2. Claim 2 has been amended, as suggested by the Examiner, to replace "in need thereof" with "diagnosed with prostate cancer and requiring treatment for said cancer". Withdrawal of the formal rejection is respectfully requested.

VI. THE 35 U.S.C. §112, FIRST PARAGRAPH, REJECTIONS

Claims 1-3 and 5-10 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly not in compliance with the written description and enablement requirements. The rejections are respectfully traversed.

The Action asserts that the claims lack adequate written description to reasonably convey that the inventors were in possession of the invention at the time of filing the application, and that the specification is not enabling for the breadth of what is claimed. The amendments to claims 1 and 2 are believed to overcome both of these rejections.

The Action also asserts that the claims are overly broad in scope as they relate to the treatment of any prostate cancer cells. However, the specification only describes the inhibition of proliferation of two out of three prostate cancer cell lines tested (LNCaP and PC-3, but not DU-145). Claims 1 and 2 are limited to prostate cancer cells that express sPLA₂-IIA to better reflect the compounds of the invention, which inhibit prostate cancer cells expressing sPLA₂-IIA. LNCaP is an androgen-dependent cell line and PC-3 is an androgen-independent cell line, and both express sPLA₂-IIA. The compounds of the invention therefore work on both androgen-dependent and androgen-independent cancer cells that express sPLA₂-IIA. DU-145 cells do not express sPLA₂-IIA, hence the negative result. Furthermore, DU-145 cells are clonal and so not necessarily representative of prostate cancer.

The Action further asserts that as the experiments in the specification were only carried out *in vitro*, it is not assured that the inhibitors will have the same effect *in vivo*, and thus the claims are overly broad. In response, attention is directed to the attached poster abstract showing that the cyclic peptides can not only slow growth of cells, but can also cause complete regression of tumors in mice *in vivo*. The animals in which this occurred remained cured for up to 3 months following treatment withdrawal. As

acknowledged in the Action, Applicants showed in *Sved et al.* that the inhibitors of the invention work *in vivo*.

Furthermore, the prior art relied upon in the Action to substantiate that xenograft models of cancer are not predictive of the situation *in vivo* all relate to cancers *other than human prostate cancer* (Action, pages 18 and 27-30; citations by *Kelland et al.*, *Gura*, *Dennis*, *Schuh*, *Bibby*, *Peterson* and *Saijo*). Each of these citations is addressed below.

Kelland et al. cites data that support a relationship between outcomes in xenograft models and clinical outcome for cytotoxic drugs. In particular, Section 2, page 829, column 1, third paragraph of *Kelland et al.* states that "overall the five drugs induced remissions in 24% (45/187) of the xenograft studies whereas minor regressions or no change occurred in 13% of cases while 63% (117/187) of xenografts progressed on treatment. These findings are similar to the overall response rates recorded for monotherapy clinical trials with these agents." The attached abstract shows similar response rates (13-27% remission) in the xenograft model to these drugs, even though it is not a cytotoxic drug. Taken together, the data support the position that in this case, Applicant's model will be predictive of clinical response.

The *Gura* article is also not relevant. First, while xenograft results may appear "not much better" than the original models, they nonetheless were as good. Second, because the compounds the Applicant used to establish the principle in this case are directed to inhibition of the human sPLA₂-IIA enzyme, there is no rationale for believing they would be "good mouse drugs". In fact, this argument argues that they are likely to be *more effective* in the clinic than in the mouse because they will inhibit stromally-

derived sPLA₂-IIA as well as the tumor derived enzyme in humans. The data in this case then also argue against the premise raised in *Dennis* that "many more (drugs) that show positive results in mice have had little or no effect in humans, possibly because the human tumours are growing in a foreign environment."

Schuh acknowledges that mice remain "the most scientifically and economically powerful model of malignant neoplasms" (see Abstract, last line). Thus, they are the best available to the art at present.

Regarding *Bibby*, the Action misinterprets the methodology used in orthotopic transplantation, indicating they have little technical skill in the art. The article acknowledges that "As yet, although a body of literature has amassed on the technique itself and its implications for metastasis, there are relatively few laboratories using these test systems in drug development programmes." The relevance of the *Bibby* citation is therefore questionable given that the model appears to be not well validated.

The comments in relation to the *Kelland et al.* citation also apply to the *Peterson* citation.

Regarding *Saijo*, the reasons cited in this article for failure of clinical trials (e.g., molecular target for each drug undetermined, target tumor uncertain, Table 6) are very unlikely to be relevant to this particular case because the Applicant has disclosed both *in vitro* and *in vivo* data that argues *against* their relevance.

Thus, the prior art cited by the Examiner in relation to the alleged inadequacy of the xenograft models is not relevant to the present case as it says nothing about models of *human prostate cancer*. As discussed above, it is currently considered in the field that xenograft models are predictive of *human prostate cancer*. One of ordinary skill

could therefore reasonably rely upon the *in vitro* data in support of the presently claimed invention without the need for human clinical trial data.

Objection is made that the definition of the "conformationally constrained molecule" of claim 6 is too broad and that these inhibitors should be limited to peptide inhibitors. In response, it is well-known in the field that peptide drugs can be turned into non-peptide drugs (called 'peptide mimetics', or 'peptidomimetics'), for example, by replacing only one peptide bond (see specification, pages 18-20 for a discussion). Furthermore, there is a definition in the specification for the term "conformationally constrained molecule" (see page 17) and these molecules are discussed at length on pages 17-22 of the specification. Thus, it would be clear to one of ordinary skill in the art which molecules are encompassed by the term "conformationally constrained molecule".

The Action objects to claims 6-9 on the ground that it is not predictable that the cyclization of a peptide will improve the potency of a sPLA₂-IIA peptide inhibitor. In response, the inventive step in the present case is the specificity encoded by the peptide sequence, as described in claim 6. This defines the class of compounds the claims seek to protect for use in the treatment of prostate cancer. Once the peptide sequence is known and in this case, given that it is known that cyclisation improves potency, it is routine (i.e. while it takes work, the work does not involve an inventive step) in the art to predict analogues that are effective, as was done with cyclisation.

In response to the comments at page 34, paragraph 1 of the Action, it is well known in the art that non-naturally occurring amino acids can be substituted for naturally occurring amino acids. Applicants have reduced this to practice by developing the

c(2NapA) analogue of cFLSYR. To repeat this process would be a matter of routine in the drug development field.

In addition, the data in *Thwinn et al.* (cited at page 34 of the Action) is not directly relevant to this case because the paper describes a first-round screen for peptide inhibitors. The publicly available art with respect to these inhibitors is much further advanced than the example given in *Thwinn et al.* Applicants describe in *Church et al.* the features of the compound structure that are important for function, thus minimizing the risk cited by *Thwinn et al.*

Regarding the comments on page 35 of the Action relating to dosages, the Applicants show in *Sved et al.* that the potency of cFLSYR (10 mg/kg sc) and c(2NapA)LS(2NapA)R (1 mg/kg) reflect the order of potency of the two compounds in inhibiting sPLA₂ enzyme activity, as shown in *Church et al.* Applicants have also shown that the linear peptide FLSYK is as potent as FLSYR. Furthermore, Applicants have data (unpublished) that cyclic FLSYK is as potent as cFLSYR in activity assays. Thus, it is reasonable to expect that cFLSYK will be effective.

With regard to the toxicity arguments on page 35 of the Action, Applicants have found that cFLSYR is not toxic even at 1000 uM. 100 uM effective dose is much higher than the effective dose (1-10 nM) the Applicants see in prostate cancer cells in culture. Thus, the potential non-toxic therapeutic window may be up to 5 orders of magnitude.

For all of the above reasons, it is believed that the 35 U.S.C. §112, first paragraph, rejections should be withdrawn. Such action is respectfully requested.

GRAHAM et al
Appl. No. 10/517,256
January 16, 2009

VII. THE ANTICIPATION REJECTION

Claims 1-3 and 5-10 stand rejected under 35 U.S.C. §102(a) as allegedly anticipated by Sved *et al.*, Cancer Res. 2004 Oct. 1; **64**: 6934-6940 (Sved). That rejection is respectfully traversed.

Sved was published after the priority date of the present application. It is clear from the comments presented above that the claims as amended are entitled to the priority date, and are not anticipated by Sved. Withdrawal of the anticipation rejection is respectfully requested .

VIII. THE OBVIOUSNESS REJECTIONS

Claims 1-3 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Graff *et al.*, Clin. Cancer Res. 2001 Dec; **7**: 3857-3861 (Graff) in view of Attiga *et al.*, Cancer Res. 2000 Aug. 15; **60**: 4629-4637 (Attiga), Liu *et al.*, J. Urol. 2000 Sept; **164**: 820-825 (Lui) or Kelavkar *et al.*, Carcinogenesis, 2001 Nov; **22** (11): 1765-1773 (Kelavkar). Claims 5-10 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Graff in view of Attiga, Liu or Kelavkar and further in view of Church *et al.*, J. Biol. Chem, 2001 Aug 31; **276** (35): 33156-33164. The rejections are respectfully traversed.

Graff suggests that increased levels of sPLA₂-IIA in the serum of patients with prostate cancer is sufficient to know that inhibition of this enzyme will be a useful treatment for prostate cancer. However, elevated levels of a biological agent in a disease state do not necessarily indicate that their inhibition will provide a suitable treatment. For example, it is known that PSA levels are elevated in prostate cancer, but

GRAHAM et al
Appl. No. 10/517,256
January 16, 2009

PSA is not considered a therapeutic target for patients with prostate cancer. It is also known that C-reactive protein levels are elevated in the serum in inflammatory disorders such as rheumatoid arthritis (RA), but C-reactive protein is not currently considered a target for treatment of RA. Elevated PLA₂ levels (in RA and sepsis) have been used as a basis for trialing PLA₂ inhibitors, but the inhibitors have failed to provide a suitable treatment for such diseases. A person of ordinary skill in the art would therefore not have had a reasonable expectation of developing a successful treatment for prostate cancer based on Graff. To arrive at a treatment for prostate cancer, the skilled artisan would first have had to identify sPLA₂-IIA inhibitors that stop proliferation of prostate cancer cells, as disclosed in the present application. In addition, Graff provides no specific examples of inhibitors of sPLA₂-IIA that are suitable for treating prostate cancer. Graff therefore provides no enabling disclosure or suggestion of the present invention as claimed.

The deficiencies of Graff are not cured by the secondary references. Thus, with regard to Attiga, it was well known at the priority date of the present application that the inhibitor used in Attiga, namely 4-bromophenacyl bromide (also known as p-bromophenacyl bromide or BPB), is a non-selective inhibitor (see attached abstracts, particularly number 1 which states that this inhibitor "should not be used as a selective inhibitor of enzymes in crude cellular experiments"). Based on this, it is clear that a person of ordinary skill in the art would not have been motivated to use inhibitors of PLA₂ to reduce or inhibit proliferation of prostate cancer cells *with a reasonable expectation of success*. The combined disclosures of Graff and Attiga do not give rise to a *prima facie* case of obviousness.

Liu is likewise irrelevant. Liu discloses cyclooxygenase (COX-2) inhibitors and their use in the treatment of prostate cancer. The COX-2 enzyme lies downstream of sPLA₂-IIA, and the Action asserts that it would therefore have been obvious to a person skilled in the art to inhibit an enzyme lying upstream of COX-2 for the treatment of prostate cancer. In response, there is no guarantee that inhibiting sPLA₂-IIA would produce the same effect as inhibiting COX-2. Both enzymes lie in a complex biochemical pathway regulated by numerous and different molecules. Hence, a skilled addressee could not reasonably predict that inhibiting sPLA₂-IIA would produce a similar effect as COX-2 inhibition. Furthermore, due to differential regulation, there would likely be different side-effects associated with the inhibition of the enzymes, since they are involved at different points within the metabolic pathway. Liu shows that blockade of COX-2 can slow proliferation of PC-3 cells and slow the growth of PC-3 xenografts. Blockade of COX-2 alone does not rule out COX-2 independent effects of the inhibitors of the present invention. The potency of inhibition of proliferation with the compounds of the present invention (1-10 nM) compared to NS398 (10-100 μM) (see Liu, Fig 1B) suggests sPLA₂ blockade in working on COX-2-independent pathways because NS398 is known to be a potent (sub-micromolar) inhibitor of COX-2 enzyme activity and PGE₂ production in cells in culture. Thus, the link made between COX-2 inhibition and sPLA₂ inhibition with Liu is tenuous.

Kelavkar discloses 15-lipoxygenase-1 (LOX) inhibitors and their use in the treatment of prostate cancer. The LOX enzyme also lies downstream of sPLA₂-IIA. Please, as with Liu, it would not have been predictable to one of ordinary skill that inhibiting sPLA₂-IIA would have the same effect as inhibiting LOX. Further, Kelavkar

GRAHAM et al
Appl. No. 10/517,256
January 16, 2009

has to over express 15 lipoxygenase-1 in PC-3 cells to see functional effects because they show (Fig 1) that PC-3 cells do not express the enzyme normally. The effects the inhibitors of the present invention have on PC-3 cells thus cannot be due to 15 lipoxygenase-1 expression, as implied in the Action. Kelavkar is therefore not relevant to Applicant's data, but rather points more to independent effects of sPLA₂ inhibition over the effects seen in Kelavkar.

In relation to both Liu and Kelavkar, it should be further noted that there are several COX and LOX enzymes (e.g., 5 lipoxygenase, 15 lipoxygenase-2, cyclooxygenase-1 12-lipoxygenase). Thus, these two citations alone would be insufficient to rule out additional beneficial effects of PLA₂ blockade mediated by these enzymes. There are also many different types of PLA₂ enzyme, and thus it would not have been obvious to one skilled in the art to choose sPLA₂-IIA for inhibition with the expectation that it would have the same effect as COX or LOX inhibition.

With regard to the obviousness rejection of claims 5-10 over the above references and further in view of Church, Church discloses compounds which are inhibitors of sPLA₂-IIA. However, there is no suggestion in Church that these compounds would be suitable for use as a treatment for prostate cancer. Furthermore, the last paragraph of Church suggests that the compounds disclosed to be used as a treatment for **inflammation**. Church therefore leads **away** from use of the compounds in the treatment of prostate cancer.

For all of the above reasons, it is clear that Graff taken alone or in combination with the cited secondary references does give rise to a *prima facie* case of obviousness. Withdrawal of the obviousness rejections is respectfully requested.

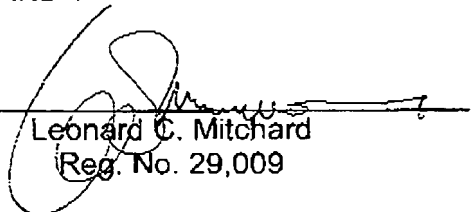
GRAHAM et al
Appl. No. 10/517,256
January 16, 2009

Favorable action on this application is awaited.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____


Leonard C. Mitchard
Reg. No. 29,009

LCM:iff
901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

Attachments: *Belinski et al.*, *Tomlinson et al.*, poster abstract

Mol Carcinog. 2007 Feb;46(2):106-16. Click here to read Links

Expression of secretory phospholipase A2 in colon tumor cells potentiates tumor growth.

Belinski GS, Rajan TV, Saria EA, Giardina C, Rosenberg DW.

Center for Molecular Medicine, The Neag Comprehensive Cancer Center, University of Connecticut Health Center, Farmington, Connecticut 06030-3101, USA.

Secretory phospholipase A2 (sPLA2-IIA) has been shown to attenuate intestinal tumorigenesis in Apc(Min) mice, demonstrating that it is a tumor modifier. To further explore the actions of sPLA2-IIA in tumorigenesis, sPLA2-IIA was overexpressed in two cell lines where it is normally absent, the murine colon tumor cell line AJ02nm0, and human colon carcinoma cell line HCT-116. Two allelic variants of sPLA2-IIA were tested in this study; sPLA2-IIA(AKR) and sPLA2-IIA(SWR), which are derived from AKR/J and SWR/J mice, respectively, and differ by a single amino acid at position 63 in the calcium- and receptor-binding domain. There was no change in cell-doubling time for either allele when compared to vector controls. Furthermore, sodium butyrate and arachidonic acid (AA)-induced cell death were unchanged in control and transfected cells. Addition of the sPLA2 substrate, palmitoyl-arachidonoyl-phosphatidic acid (PAPA), to AJ02nm0 cells resulted in a modest (12%-24%), but significant ($P < 0.01$), inhibition of growth that was dependent on sPLA2-IIA expression. However, when AJ02nm0 and HCT-116 cells were injected subcutaneously (sc) into nude mice, Pla2g2a expression resulted in a 2.5-fold increase in tumor size. In addition, sPLA2-IIA expressing HCT-116 tumors were found to be more infiltrative than controls. We conclude that the ability of sPLA2-IIA to slow tumor cell growth is dependent upon the availability of substrate, and that in some instances sPLA2-IIA may actually enhance tumor growth. Mechanisms that may account for differences between the tumor explant model versus the Apc(Min) model of intestinal cancer are discussed. 2006 Wiley-Liss, Inc. 1: Ann Hum Genet. 1996 Sep;60(Pt 5):369-76. Links

Variants at the secretory phospholipase A2 (PLA2G2A) locus: analysis of associations with familial adenomatous polyposis and sporadic colorectal tumours.

Tomlinson IP, Beck NE, Neale K, Bodmer WF.

Cancer Genetics Laboratory, Imperial Cancer Research Fund, London, UK.

The Min mouse is a model for human familial adenomatous polyposis (FAP), an autosomal dominant disease characterised by multiple adenomatous gastrointestinal polyps. The severity of the Min phenotype is modified by a locus (Mom1) on mouse chromosome 4, at a position syntenic with human chromosome 1p35-p36. The secretory phospholipase A2 (Pla2s) gene is a candidate for this modifier locus and there is evidence that a locus on human chromosome 1p35-p36 acts to modify the severity of human duodenal FAP. We have analysed the human secretory phospholipase A2 locus (PLA2G2A) for variants that could directly influence the FAP phenotype. We found no PLA2G2A variants predicted to result in functional variation in the phospholipase A2 protein. Two PLA2G2A polymorphisms were, however, discovered, one a 'silent' base change in exon 3 and another in a noncoding region. Three other variants (possible mutations) were found in non-coding regions. In 70 FAP patients from 20 families, no associations were found between the severity of duodenal polyposis and any PLA2G2A variant. One allele at the exon 3 polymorphic site did, however, occur more often than expected in patients with relatively severe colonic FAP. Although of borderline statistical significance, this association, if genuine, is likely to result from linkage disequilibrium between the PLA2G2A alleles studied and undetected genetic variation at a closely linked locus. The frequency of the alleles at both polymorphic sites has also been determined in the germ line of patients with sporadic colorectal adenomas and carcinomas and in random controls, but no differences were found among these groups. Our results suggest that PLA2G2A variants do not influence inherited or sporadic colonic tumours. A linked locus may be a modifier of human FAP, but does

not influence the risk of colorectal tumours in the
general population.

Abstract ID: ABSNS-YT7G6-2EXZ3-KJPL6

Regression of aggressive advanced prostate cancer, in a xenograft model, upon oral administration of cyclic pentapeptide, secreted phospholipase A₂ inhibitors.

K. F. Scott¹, M. Sajinovic², A. Qadir³, V. Kumar³, G. Cooney⁴, Q. Dong⁵, W. Liauw⁶, G. G. Graham⁷, P. J. Russell^{2,8}

¹ St Vincent's Hospital Clinical School, The University of New South Wales, Sydney, NSW, Australia

² Oncology Research Centre, Prince of Wales Hospital, Sydney, NSW, Australia

³ School of Medical Sciences, The University of New South Wales, Sydney, NSW, Australia

⁴ Diabetes and Obesity Research Programme, Garvan Institute of Medical Research, Sydney, NSW, Australia

⁵ Department of Medicine, The University of Sydney, Sydney, NSW, Australia

⁶ Cancer Care Centre, St George Hospital, Sydney, NSW, Australia

⁷ Department of Clinical Pharmacology, St Vincent's Hospital, Sydney, NSW, Australia

⁸ Prince of Wales Clinical School, The University of New South Wales, Sydney, NSW, Australia

Current therapy for advanced or metastatic prostate cancer (PC) is androgen ablation by surgical or pharmacological means. For hormone refractory PC (HRPC) chemotherapy (docetaxel and prednisone) offers a modest (2.4 month) median survival advantage compared to the previous standard of care, but the overall median survival is still only 18.9 months¹. We have identified and validated Group IIA secreted phospholipase A₂ (hGIIA) as a novel target for HRPC therapy². hGIIA remains constitutively expressed in PC tissues following 3 months androgen ablation therapy. The increased hGIIA has an oncogenic action and our novel hGIIA inhibitors cFLSYR (cF) and c(2NapA)LS(2NapA)R (c2) slow androgen-insensitive xenograft (PC-3) tumour growth in immunodeficient mice on subcutaneous (sc) administration². Here we show, using [³H]-leucine-labelled compounds, that both are absorbed upon single-dose oral administration to BALB/c mice, achieving peak plasma levels comparable to sc administration (cF) or 50% peak sc levels (c2). Both compounds distribute to tissues including liver, kidney and brain. HPLC analysis of liver extracts shows that both peptides remain intact on sc administration. Upon repeated oral administration, both c2 (p<0.001, 1-way ANOVA, Bonferroni's multiple comparison test) and cF (p<0.05) slowed the growth of established tumours (PC-3M-luc in BALB/c nu/nu mice) relative to vehicle controls. Some animals (c2, 4/15, 27%; cF, 2/15, 13%), survived until study termination at six months, while all control animals were euthanased due to excessive tumour size by 2.25 months. Three survivors showed a decline from peak tumour volume over six months treatment. The remaining three survivors showed no measurable disease by three months. Treatment was withdrawn one week post-tumour regression in each case and no tumour recurred in these animals for the remainder of the study. These data indicate that clinical studies are warranted to determine the safety and benefit of these compounds as oral treatments for HRPC.

Abstract ID: ABSNS-YT7G6-2EXZ3-KJPL6

(1) Tannock, IF et al. N. Engl. J. Med. 351:1502, 2004.

(2) Sved, Scott et al. Cancer Res. 64:6934-6940, 2004.

(3) This work has been supported by the Cancer Council, NSW, (KFS, GG, QD, PJR), a DVA grant (QD, GG, KFS, PJR) and NHMRC Grant 222870 (KFS, GG, HPMcNeil).

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Result 1.

Nonspecific inhibition of enzymes by p-bromophenacyl
bromide. Inhibition of human platelet phospholipase C
and modification of sulfhydryl groups.

Kyger EM, Franson RC

Link to...

- * Abstract
- * Complete Reference

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Unique Identifier 6733133

Status MEDLINE

Authors Kyger EM. Franson RC.

Authors Full Name Kyger, E M. Franson, R C.

Title Nonspecific inhibition of enzymes by p-
bromophenacyl bromide. Inhibition of human platelet
phospholipase C and modification of sulfhydryl
groups.

Source Biochimica et Biophysica Acta. 794(1):96-
103, 1984 Jun 6.

Abstract This study demonstrates that p-
bromophenacyl bromide irreversibly inhibits, in a
time- and dose-dependent manner, yeast alcohol
dehydrogenase, bovine pancreatic alpha-chymotrypsin,
human platelet phosphatidylinositol (PI)-specific
phospholipase C, in addition to the neutral-active
and calcium-dependent phospholipase A2 of human
platelets. The PI-specific phospholipase C has
maximal activity at pH 5,5 is calcium-dependent, and
is strongly inhibited by sulfhydryl reagents 5,5'-
dithiobis(2-nitrobenzoic acid) (DTNB) and
methylmethane thiosulfonate . Increasing
concentrations of DTNB produced concomitant
inhibition of phospholipase C activity and titration
of sulfhydryl groups. In contrast, human platelet

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phospholipase A2 activity was unaffected by concentrations of DTNB that titrated sulfhydryl groups, and completely inhibited PI-specific phospholipase C activity. Treatment of cysteine with p-bromophenacyl bromide resulted in modification of the amino acid as demonstrated by paper chromatography, and loss of titratable sulfhydryl groups. These data show that p-bromophenacyl bromide inhibits a wide spectrum of enzymatic activities including PI-specific phospholipase C. This reagent modifies amino acid residues other than active-site histidines and therefore has a broader reactivity than previously considered. Thus, it should not be used as a selective inhibitor of enzymes in crude cellular experiments.
Publication Type Journal Article. Research Support, U.S. Gov't, P.H.S..

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Results Available: 2

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Result 1.

Effect of putative phospholipase A2 inhibitors on acetic acid-induced acute colitis in the rat.

Fabia R, Ar'Rajab A, Willen R, Andersson R, Bengmark S

Link to...

* Abstract

* Complete Reference

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Unique Identifier 8402133

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Authors Fabia R. Ar'Rajab A. Willen R. Andersson R. Bengmark S.

Authors Full Name Fabia, R. Ar'Rajab, A. Willen, R. Andersson, R. Bengmark, S.
Institution Department of Surgery, Lund University, Sweden.

Title Effect of putative phospholipase A2 inhibitors on acetic acid-induced acute colitis in the rat.

Source British Journal of Surgery. 80(9):1199-204, 1993 Sep.

Abstract Phospholipase activation may play an important role in ulcerative colitis. This hypothesis was tested by evaluating the effect of two non-selective phospholipase (PL) A2 inhibitors, quinacrine and p-bromophenacyl-bromide (pBPB), on acetic acid-induced colitis in the rat. The calcium antagonist verapamil, which may also act as a PLA2 inhibitor, was also tested. Acute colitis was induced in an isolated colonic segment by instillation of 4 per cent acetic acid for 15 s; this induces a uniform colitis after 4 days. The severity of colitis was evaluated histologically, by measuring myeloperoxidase (MPO) activity and by determining plasma exudation into the lumen of the colon (permeability) with 125I-labelled albumin given intravenously. All three putative PLA2 inhibitors tested were found to prevent the development of colitis. Intravenous administration of quinacrine 10 mg kg⁻¹ at 30 min before instillation of acetic acid resulted in a normal mucosal appearance, normal MPO activity and a significantly reduced increase in plasma exudation into the colon. A similar effect was achieved using verapamil. Intracolonic administration of either quinacrine or pBPB also prevented acetic acid-induced colitis. However, three doses, starting immediately after acetic acid administration and repeated on the first and second days, were needed to achieve this, whereas one dose produced only a partial effect. PLA2 may play an important role in acetic acid-induced colitis and inhibition of its activity may offer an alternative mode of treatment in ulcerative colitis.

Publication Type Journal Article. Research Support, Non-U.S. Gov't.

Result 2.

Phospholipase A2 inhibitors. Differential inhibition of fatty acid acylation into kidney lipids by mepacrine and p-bromophenacyl bromide.

Erman A, Azuri R, Raz A

Link to...

- * Abstract
- * Complete Reference
- * Full Text

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Unique Identifier 6870935

Status MEDLINE

Authors Erman A. Azuri R. Raz A.

Authors Full Name Erman, A. Azuri, R. Raz, A.

Title Phospholipase A2 inhibitors. Differential inhibition of fatty acid acylation into kidney lipids by mepacrine and p-bromophenacyl bromide.

Source Biochemical Pharmacology. 32(13):2083-7, 1983 Jul 1.

Abstract Mepacrine and p-bromophenacyl bromide, in addition to their inhibitory effect on lipolysis, are also potent inhibitors of fatty acid acylation into renal medullary lipids. Significant qualitative and quantitative differences in the inhibition by the two drugs were seen. p-Bromophenacyl bromide exerted a non-selective effect inhibiting the incorporation of saturated and unsaturated fatty acids into all phospholipid classes and triacylglycerols. In contrast, mepacrine selectively inhibited the incorporation of both saturated and unsaturated acids into phosphatidylcholine, phosphatidylethanolamine and triglycerides, and concurrently markedly enhanced their incorporation into phosphatidylinositol. Quantitative analysis of these mepacrine effects, together with the known inhibitory effects of this compound on phospholipase A2 and phosphatidylinositol-specific phospholipase C, suggests that mepacrine also inhibits phosphatidic acid phosphatase, thereby shunting the flux of phosphatidic acid away from diglyceride formation and into synthesis of phosphatidylinositol.

Publication Type Comparative Study. In Vitro. Journal Article.

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Version: OvidSP_UI01.02.01, SourceID 36463